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INTERACTIONS BETWEEN BLOW FLIES (CALLIPHORIDAE) AND *ENTOMOPHTHORA BULLATA* (PHYCOMYCETES: ENTOMOPHTHORALES)

John Paul Kramer

Abstract.—Studies of interactions between blow flies and *Entomophthora bullata* showed that: (1) the time between exposure to conidia and death of the fly ranged from 5 to 12 days with about 50% dying by day 6; (2) the fungus produced only conidia in 79% of the cadavers, resting spores in 18%, and a mixture of the two in 3% of them; (3) in flies ½ to 3 days old at exposure only conidia were formed in >80% of the cadavers, while in flies 4 to 5 days old at exposure only resting spores were formed in about 50% of the cadavers; (4) the death rates of flies were not influenced by their ages at exposure; and (5) the time required for the disease to kill flies was not related to the types of spores formed on or in their cadavers. Host species included *Phormia regina, Phaenicia sericata,* and *Protophormia terraenovae.*

Introduction

On August 10, 1973, I found two fresh cadavers of black blow flies, *Phormia regina,* that had apparently succumbed to a mycosis in a cage of miscellaneous muscoids taken alive two days earlier from a trap set in a wooded area near Ithaca, New York. I tied both cadavers to the wet wick of a water bottle which rested on a layer of moist sand within a glass battery jar. The jar, covered with clear polyethylene wrap to maintain a relative humidity close to saturation, was held overnight in a dark and cold room (14–16°C). By the following morning a reddish tan mycelial mat had grown over one cadaver; only the dorsum of its thorax remained uncovered. A thick layer of conidia discharged from this cadaver covered the aluminum foil collar of the water bottle and portions of the wick as well. The body surface of the second cadaver exhibited no growths and this specimen was removed from the wick for further study. A sample of the conidial shower produced by the first cadaver was also removed from the wick for microscopical examination.

The body cavity of the second cadaver was filled with spherical and hyaline bullate resting spores that ranged from 38 to 58 μm in diameter. The discharged conidia from the first cadaver were hyaline and elliptical with an evenly rounded apex and a papillate base. These conidia ranged in size from 25–37 × 11–17 μm. In conformation and size these resting spores and con-

idia compare very favorably with those described by MacLeod et al. (1973) for *Entomophthora bullata*. I therefore concluded that the fungus found in these *P. regina* was the same species.

The present study was done: (1) to develop baseline data on the *in vivo* culture of *E. bullata* and its effects on blow flies, and (2) to determine if an association exists between the age of flies at exposure to conidia and the time of their death due to *E. bullata* infections and the types of spores produced in their cadavers.

Materials and Methods

The *P. regina* cadaver that produced the conidial shower, noted in the first section of this paper, served as the source of inoculum for the infectivity tests. Thirty young, laboratory-reared *P. regina* adults were added to the battery jar containing the water bottle with this cadaver tied to its wick. As noted previously, the collar of the water bottle and portions of the wick were covered with a thick layer of conidia. The jar containing this contaminated bottle and the healthy flies was returned to the dark and cold room. On the following morning the jar was placed on a laboratory bench at room temperatures (21–27°C). Here the flies received some artificial light from overhead fluorescent lamps during each day for the duration of the experiment. A small dish of dry sugar-milk was added to the jar as a source of nourishment for the flies. Several small holes were punched in the polyethylene cover for ventilation. The flies were observed at daily intervals and dead ones were removed from the jar and examined for signs of *E. bullata* infections. The data pertaining to this specific test are presented in Table 1 (see Test 1). The same procedures were used in all of the subsequent infectivity tests. All of the *P. regina, Phaenicia sericata,* and *Protophormia terraenovae* tested came from clean laboratory cultures.

The results given in Tables 2 through 4 are pooled data from 49 separate tests. The number of flies used in any single test was variable and depended upon the number of flies available at the time. While records on the ages of flies were maintained, records on the sex ratios within the test groups were not. Flies that died without *E. bullata* infections and flies that lived for more than 12 days following exposure to *E. bullata* conidia have been omitted from the data presented in Tables 2 through 4. No flies living for more than 12 days died with *E. bullata* infections.

Results and Interpretations

The data in Table 1 show that 27 serial passages of *E. bullata* were achieved from August 1973 to March 1974. The failure to effect transmission for additional passages was linked to a bacterial septicemia found in most

Table 1. Fly-to-fly passages of *Entomophthora bullata* in adult blow flies during an eight-month period under laboratory conditions.

Test number	Date test started	Flies tested		No. cadavers as inoculum sources*	Percentage dying with *E. bullata***
		Species	Age (days)		
1	13 Aug.	*P. regina*	1/2	1	79 (23/30)
2	20 Aug.	*P. regina*	4	2	40 (6/15)
3	28 Aug.	*P. regina*	1/2	2	22 (4/18)
4	5 Sep.	*P. regina*	5	1	11 (3/27)
5	13 Sep.	*P. regina*	1/2	1	44 (10/23)
6	20 Sep.	*P. regina*	1/2	1	31 (6/18)
7	26 Sep.	*P. sericata*	1/2	1	53 (8/15)
8	4 Oct.	*P. regina*	1/2	2	100 (17/17)
9	10 Oct.	*P. terraenovae*	1/2	2	100 (10/10)
10	17 Oct.	*P. sericata*	5	1	86 (12/14)
11	26 Oct.	*P. regina*	1/2	1	59 (17/29)
12	5 Nov.	*P. sericata*	1/2	1	74 (17/23)
13	12 Nov.	*P. regina*	1/2	3	92 (12/13)
14	21 Nov.	*P. sericata*	1/2	1	79 (11/14)
15	28 Nov.	*P. regina*	1/2	3	82 (28/34)
16	5 Dec.	*P. sericata*	1/2	1	58 (11/19)
17	13 Dec.	*P. regina*	1/2	3	83 (15/18)
18	26 Dec.	*P. sericata*	1/2	1	100 (10/10)
19	2 Jan.	*P. regina*	1	1	67 (8/12)
20	14 Jan.	*P. regina*	1	1	29 (2/7)
21	25 Jan.	*P. regina*	1	1	44 (4/9)
22	4 Feb.	*P. sericata*	1	1	54 (15/28)
23	12 Feb.	*P. regina*	1	5	40 (15/28)
24	21 Feb.	*P. regina*	3	3	67 (4/6)
25	6 Mar.	*P. sericata*	1	1	37 (7/19)
26	19 Mar.	*P. sericata*	1	1	6 (1/18)
27	27 Mar.	*P. regina*	1	1	50 (5/10)
28	4 Apr.	*P. sericata*	2	3	0 (0/22)

* Source for Test 1 was a field-collected *P. regina;* sources for subsequent tests were conidia-producing cadavers from the previous trial.

** By post-exposure day 12.

of the *E. bullata*-infected flies that died in Tests 23 through 27 (see Table 1) and this severely depressed the development of the fungus. Hence the numbers of infectious units to which the flies were exposed declined during these last five tests. The data in Table 1 show that the percentage of flies dying with *E. bullata* infections ranged from 6 to 100%, yet there is no obvious relationship between the numbers of cadavers used as inoculum sources and the percentages of flies that acquired the infection. In nearly all cases the cadavers were attached to the substrate by rhizoids. All three species of blow flies were susceptible to infection.

Table 2. Age of blow flies at time of exposure to *Entomophthora bullata* conidia and time of death due to infection.

Age in days		(No.)	Post-exposure days and percentage dying during each period								
			1–4	5	6	7	8	9	10	11	12
½ to 1	*P. regina*	(454)	0	14	45	15	14	6	4	0.5	1.5
	P. sericata	(225)	0	19	40	28	7	4	1.5	0.5	0
	Both spp.	(679)	0	16	43	19	11	5	4	0.5	1.5
2	*P. regina*	0	0	0	0	0	0	0	0	0	0
	P. sericata	(16)	0	0	0	56	0	13	25	0	6
	Both spp.	(16)	0	0	0	56	0	13	25	0	6
3	*P. regina*	(46)	0	0	23	43	24	4	2	2	2
	P. sericata	(14)	0	57	43	0	0	0	0	0	0
	Both spp.	(60)	0	13	27	33	18	3	2	2	2
4	*P. regina*	(18)	0	6	11	33	28	0	11	11	0
	P. sericata	(34)	0	12	34	29	17	3	6	0	0
	Both spp.	(52)	0	10	25	31	21	2	8	3	0
5	*P. regina*	(18)	0	0	83	17	0	0	0	0	0
	P. sericata	(14)	0	0	86	0	0	0	7	7	0
	Both spp.	(32)	0	0	84	8	0	0	4	4	0
All ages in both species		(839)	0	14	42	21	12	5	4	1	1

Flies died of *E. bullata* infections as early as day 5 and as late as day 12 following exposure to conidia with about 50% dying by day 6 and 75% by day 7 (see Table 2). The data presented also suggest that susceptibility to infection among flies ½ to 5 days old is about the same in all of these age classes, and in both species tested. The data on age classes presented in this table, however, do not suggest that age of the fly at exposure alters the time required for the disease to kill the fly, i.e. older flies die at about the same rate as younger ones.

The data in Table 3 show that the fungus produced conidia in 79% of the cadavers, resting spores in 18%, and a mixture of conidia and resting spores in 3% of them. In this last group very light conidial showers were produced from scanty mycelial mats that grew on the surfaces of abdomens that harbored some resting spores internally. The data in Table 3 suggest that age of the fly at time of exposure does influence the types of spores produced by the fungus in the cadavers; in more than 80% of the flies ½ to 3 days old at exposure only conidia were formed, while in about 50% of the flies 4 to 5 days old only resting spores were formed. This observation supports the suggestion of Wilding and Lauckner (1974) that resting spore formation occurs more frequently in older wheat bulb flies, *Leptophylemyia coarctata*, infected with *E. muscae*.

Table 3. Age of blow flies at time of exposure to *Entomophthora bullata* conidia and types of spores produced in their cadavers.

Age in days		(No.)	Spore types with percentages of each		
			Conidia	Resting spores	Conidia + resting spores
½ to 1	*P. regina*	(454)	81	15	4
	P. sericata	(225)	88	9	3
	Both spp.	(679)	84	13	3
2	*P. regina*	0	0	0	0
	P. sericata	(16)	94	6	0
	Both spp.	(16)	94	6	0
3	*P. regina*	(46)	86	7	7
	P. sericata	(14)	29	71	0
	Both spp.	(60)	73	22	5
4	*P. regina*	(18)	22	78	0
	P. sericata	(34)	68	32	0
	Both spp.	(52)	48	52	0
5	*P. regina*	(18)	56	44	0
	P. sericata	(14)	21	79	0
	Both spp.	(32)	41	59	0
All ages in both species		(839)	79	18	3

Table 4. Time of death of *Entomophthora bullata*-infected blow flies and types of spores produced in their cadavers.

Spore types	(No.)	Post-exposure days and percentages dying during each period								
		1–4	5	6	7	8	9	10	11	12
Conidia										
P. regina	(423)	0	13	37	22	15	6	4	1	2
P. sericata	(242)	0	16	44	30	6	1.5	1.5	0.5	0.5
Both spp.	(665)	0	14	40	25	12	5	3	0.5	0.5
Resting spores										
P. regina	(94)	0	2	63	6	16	9	2	0	2
P. sericata	(56)	0	23	41	16	9	0	7	4	0
Both spp.	(150)	0	10	55	10	13	6	5	0.5	0.5
Conidia + resting spores										
P. regina	(19)	0	0	42	58	0	0	0	0	0
P. sericata	(5)	0	0	100	0	0	0	0	0	0
Both spp.	(24)	0	0	54	46	0	0	0	0	0
All spore types in both species	(839)	0	14	42	21	12	5	4	1	1

Conidia or resting spores may be formed in flies dying from days 5 through 12 (see Table 4). This phenomenon was observed in both *P. regina* and *P. sericata*. The conidia-resting spores mixture, found only in flies ½ to 3 days old at exposure, occurred in specimens dying on days 6 and 7 only. The data in Table 4 do not suggest that the time required for the disease to kill the fly is related to the type of spores formed on or in their cadavers.

Discussion

Prior to the discovery of *E. bullata* in *P. regina* reported in the present study, this fungus has been found parasitizing several other species of blow flies and flesh flies in nature. Povah (1935) found it in blue bottle flies and MacLeod (1956) extended its host range to include *Sarcophaga aldrichi* and a *Calliphora* species. MacLeod et al. (1973) observed an outbreak of *E. bullata* infections in a field population of *S. aldrichi*, and suggested that the life cycle of the fungus involved alternating generations of conidia and resting spores. The results of the present study, however, clearly indicate that conidia or resting spores, and in some cases a mixture of the two, may be produced on or in the dead bodies of flies that acquired the infection by exposure to conidia only. The host range of *E. bullata* in nature probably includes many species of blow flies that share the same habitat, e.g. *P. regina*, *P. sericata*, and *P. terraenovae*.

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Department of Entomology, Cornell University, Ithaca, New York 14853.

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